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EDITORIAL II

On the reversal of new oral anti-coagulants: can we simply extrapolate data from the animal models to humans?

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New oral anti-coagulant agents including direct factor Xa inhibitors (anti-Xa) and direct thrombin inhibitors (anti-IIa) are rapidly entering into clinical practice. These agents have been recently licensed in many countries for post-operative prophylaxis of thromboembolic events after total hip or knee replacement surgery, and they are becoming important options for long-term anti-coagulation therapy for venous thromboprophylaxis, or stroke prevention in non-valvular atrial fibrillation.[1] On the one hand, these drugs are a welcome change for the patients because they seem to provide reliable anti-thrombotic effects without cumbersome laboratory testing and food restrictions.[1] On the other hand, the lack of specific antidotes for these agents poses major challenges in the perioperative care and bleeding management.[2–4]

Anaesthetists and intensivists have previously learnt the difficulty of managing the balance of haemostasis and thrombosis through perioperative uses of argatroban, bivalirudin, and lepirudin.[5 6] While clinical efficacy and relative safety of new oral anti-coagulants have been demonstrated, our knowledge remains limited on the management of bleeding related to these agents.[3 4 7 8] It is thus reasonable to select a potentially useful agent by testing it in a standardized animal bleeding model for haemostatic efficacy. Indeed, there have been a number of animal studies that have examined the reversibility of anti-IIa (melagatran and dabigatran) and anti-Xa (rivaroxaban and edoxaban) using the factor concentrate, including recombinant activated factor VII (rFVIIa), factor VIII bypassing agent (FEIBA), and prothrombin complex concentrate (PCC). The models, anti-coagulants, and agents are summarized in Table 1.[9–18] Although ximelagatran has been withdrawn from market due to liver toxicity, early experimental data on melagatran (active metabolite of ximelagatran) remain pertinent as it exerts anti-IIa kinetics similar to dabigatran.[19] Understanding potential usefulness and limitations of specific experimental models and agents is important because there is a risk of thrombosis with such haemostatic intervention in high-risk patients.[20 21] Pertinent animal studies of anti-IIa and anti-Xa agents widely vary in terms of animal types, vascular injury (timing, location, and extent), and coagulation tests utilized (Table 1). These differences might have led to the mixed results in the efficacy of a specific intervention, and further limit their applicability to human conditions. For example, haemostatic activity of rFVIIa is critically dependent on exposed tissue factor (TF) at the site of vascular injury.[22] However, TF expression is a time-limited event[23] because locally accumulated platelets *in vivo* prevent TF from binding to FVII (Fig. 1). Most experimental designs involved pre-treating animals with a haemostatic agent, followed by inducing a vascular injury. It is likely that the therapeutic window for an intervention is much narrower in clinical bleeding because haemostatic agents are only administered after a variable interval after the vascular injury, and the diagnosis of haemorrhage.[3 24]

Further, species specificity for anti-coagulants and haemostatic agents is an important consideration. The dose necessary to inhibit thrombosis by 50% (ED_{50}) after oral administration of rivaroxaban differs from 0.6 to 5 mg kg^{-1} in rabbits and rats, respectively, in the arteriovenous shunt model.[25] Therapeutic responses to rFVIIa vary among species as well. The mouse requires much higher rFVIIa doses (3–10 mg kg^{-1}) compared with human and primate doses (90–270 μg kg^{-1}).[11 26] Injecting human coagulation proteins in various species may not only alter coagulation factor interactions, but also coagulation assay results. The endogenous thrombin potential could be improved when human thrombin is generated in rabbit plasma, but haemostasis was not improved *in vivo*.[17] Elevated thrombin–anti-thrombin (TAT) complex reflects procoagulant responses in the host animals after rFVIIa, FEIBA, and PCC administration,[9 10 16 18] but prothrombin time (PT) and activated prothrombin time (aPTT) are not suitable for monitoring anti-IIa and anti-Xa agents, nor for predicting therapeutic effects of haemostatic agents.[27] The latter tests also exclude dynamic interactions of platelets, coagulation factors, and inhibitors (anti-IIa or anti-Xa) which take place *in vivo*.[7 17] Lastly, the location and extent of vascular injury can be important determinants of experimental outcomes.

Table 1 Animal models, agents, and doses for anti-Xa and anti-IIa reversal. '∼', no change; '↓', decrease; '↑', increase; IU, international unit; BT, bleeding time; ICH, intracranial haemorrhage; CFR, cyclic flow reduction (in the carotid artery); PT, prothrombin time; APTT, activated partial thromboplastin time; ECT, ecarin clotting time; WBCT, whole blood clotting time; ACT, activated clotting time; TT, thrombin time; TAT, thrombin–anti-thrombin complex level; TEM, thromboelastometry; TG, thrombin generation

Model	Injury	Anticoagulant	Agent/dose	Endpoints	Reference
Rat	*Tail BT*	Melagatran	FEIBA 25–400 IU kg^{-1}	↓BT, ↓ECT, ↓PT, ↑aPTT	Elg and colleagues[9]
		0.5–2.0 μg kg^{-1} h^{-1}	rFVIIa 1–10 mg kg^{-1}	∼BT, ↓WBCT, ↓PT	
Rat	*Tail BT*	Melagatran	FEIBA 200 IU kg^{-1}	↓BT, ↓WBCT, ∼aPTT, ↑TAT	Elg and colleagues[10]
		0.8 μg kg^{-1}+2 μmol kg^{-1} h^{-1}	rFVIIa 1 mg kg^{-1}	∼BT, ↓WBCT, ∼aPTT, ↑TAT	
			PCC ∼200 IU kg^{-1}	↓BT, ↑WBCT, ↑aPTT, ↑TAT	
Rabbit	*Ear BT*	Melagatran	FEIBA 200 IU kg^{-1}	↓BT, ↓WBCT, ∼aPTT	Elg and colleagues[10]
		0.8 μg kg^{-1}+2 μmol kg^{-1} h^{-1}	rFVIIa 1 mg kg^{-1}	↓WBCT, ∼ACT, ∼aPTT	
			PCC ∼200 IU kg^{-1}	∼BT, ↑WBCT, ↑aPTT	
Baboon	*Skin BT*,	Melagatran	FEIBA 100+150 IU kg^{-1}	∼BT, ↑shunt thrombus	Gruber and colleagues[11]
	AV shunt	0.6 mg kg^{-1} h^{-1}	rFVIIa 90 + 180 μg kg^{-1}	↓BT, no effect on shunt thrombus	
Rat	*Tail BT*	Dabigatran	FEIBA 50 or 100 IU kg^{-1}	↓BT, ∼aPTT	van Ryn and colleagues[12]
		0.5 μmol kg^{-1} h^{-1} for 25 min	rFVIIa 0.1 or 0.5 mg kg^{-1}	↓BT, ↓aPTT (dose-dependent)	
Mouse	*Tail BT*,	Dabigatran	PCC 100 U kg^{-1}	↓BT, ↓ haematoma expansion	Zhou and colleagues[13]
	ICH	4.5 or 9.0 mg kg^{-1}	rFVIIa 8.0 mg kg^{-1}	No effects on BT or haematoma expansion	
			Murine plasma 200 μl	No effects on BT or haematoma expansion	
Mouse	*Tail transection*	Dabigatran	FEIBA 100 IU kg^{-1}	∼Blood loss, ↓BT, ∼aPTT	Lambourne and colleagues[14]
		60 mg kg^{-1} p.o.	rFVIIa 3 mg kg^{-1}	∼Blood loss, ↓BT, ↓aPTT	
			PCC 14.3 IU kg^{-1}	∼Blood loss, ↓BT, ∼aPTT	
			PCC 14.3 IU kg^{-1}+ rFVIIa 3 mg kg^{-1}	∼Blood loss, ↓BT, ↓aPTT, ↓TT	
Rabbit	*Kidney incision*	Dabigatran 0.4 mg kg^{-1}	PCC 20, 35, 50 IU kg^{-1}	↓Blood loss, ↓BT, ↓PT, ∼aPTT, improved TEM, ↑TG peak (dose-dependent)	Pragst and colleagues[15]
Rat	*Tail BT*	Rivaroxaban	PCC 50 IU kg^{-1}	↓BT, ↓PT, ↑TAT	Perzborn and colleagues[16]
		1.3 mg kg^{-1}			
Rabbit	*Spleen/liver incision,*	Rivaroxaban	PCC 40 IU kg^{-1}	∼Blood loss, ∼BT, ↓PT, ↓aPTT, improved TEM, ∼CFR	Godier and colleagues[17]
	Ear BT, CFR	5 mg kg^{-1}	rFVIIa 150 μg kg^{-1}	∼Blood loss, ↓BT, ↓PT, ↓aPTT, improved TEM, ↓lagtime of TG, ∼CFR	
Rat	*Skin BT*	Edoxaban	FEIBA 50 or 100 IU kg^{-1}	↓BT, ↓PT (dose-dependent)	Fukuda and colleagues[18]
		1 mg kg^{-1} h^{-1}	rFVIIa 0.3–3 mg kg^{-1}	↓BT, ↓PT, ↑TAT (dose-dependent), no increased venous thrombus	

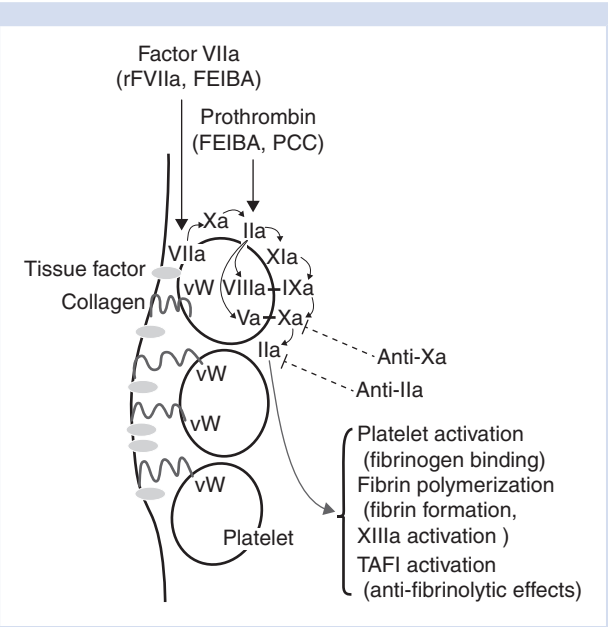

Fig 1 After the vascular injury, TF and collagen are locally expressed. TF binds to activated factor VII (VIIa) in plasma, and generates activated factor X (Xa), and subsequently trace amounts of thrombin (IIa). Platelet adhesion to collagen via von Willebrand factor (vW) covers the injury site, physically limiting TF activity. The subsequent procoagulant responses are thus driven by thrombin-mediated activation of intrinsic tenase (IXa–VIIIa) and prothrombinase (Xa–Va) on the activated platelet surface. rFVIIa and FEIBA can augment the initial amount of VIIa for early thrombin generation. FEIBA and PCC supply more prothrombin to increase thrombin generation on the activated platelet surface. Anti-Xa agents (apixaban, edoxaban, rivaroxaban, etc.) inhibit Xa and Xa–Va complex, and thrombin generation on the platelet surface is obtunded. Anti-IIa agents (argatroban, dabigatran, etc.) inhibit IIa, and prevent ongoing platelet activation, fibrin polymerization, and clot stabilization (e.g. activation of thrombin-activated fibrinolysis inhibitor; TAFI).

PCC concentrates (50–100 IU kg^{-1}) have been shown to be effective against dabigatran by reducing murine intracranial haematoma expansion induced by collagen injection,[13] and decreasing bleeding after kidney incision in rabbits.[15] However, PCC (40 IU kg^{-1}) and rFVIIa (150 μg kg^{-1}) failed to decrease bleeding from liver and splenic incisions in rivaroxaban-treated rabbits.[17] FEIBA (250 IU kg^{-1}), but not rFVIIa (270 μg kg^{-1}), increased the deposition of thrombus in the arteriovenous vascular shunt in melagatran-treated animals.[11]

In conclusion, pre-clinical animal data on haemostatic agents for the reversal of anti-Xa and anti-IIa drugs are encouraging, but they fail to provide sufficient information on the optimal timing, dose(s), and efficacy in humans. Given the lack of clinical evidence of haemostatic agents for anti-IIa and anti-Xa reversal in a variety of clinical scenarios,[28 29] the need for rFVIIa, FEIBA, or PCC for patients with uncontrolled bleeding must be individually assessed by the treating physician according to the institutional transfusion guideline and the available consensus.[30] It is also important to combine other available measures (e.g. activated charcoal, dialysis, topical haemostatics) with i.v. agents, so that the dose requirement can be minimized for the latter.

Declaration of interest

None declared.

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